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A QUALITATIVE CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF BIOLOGICAL FACTORS

EHRENFRIED E. PFEIFFER

For the reader who is disinclined to "work his way through" a scientific paper, it can be suggested that the colored illustrations in the four-page insert tell the story in its essentials.

ED. B.D.

I. Differences in Humus and Compost Quality

METHOD:

Since 1953, a simple chromatographic method has been used in the laboratory of the author for the purpose of determining differences in the formation of humus in soils, as well as in compost, differences which cannot be determined by chemical methods of analysis. There are soils which have almost identical values of available mineral substances; their biological efficiency, however, as well as the yield and quality of the crops grown on such soils differ widely. Quite a few years ago, we published the analyses of two soils which were nearly identical. The one field produced maximum yields, the other far below average, medium yields. The difference between the two lay in the soil structure and the humus condition. In a similar way, composts containing the same mineral substances, the same NPK, the same pH, even the same amount of organic substance, may have a widely varying biological effect upon soil structure, humus formation and humus condition, upon yield, germinating quality of the seeds, and upon protein quality.

In order to differentiate between these qualitative and biological values, the method to be described here was developed. It rests upon the property of certain specially manufactured filter papers, through which individual fractions may be separated, which, in turn, may be made visible through a reagent. This qualitative, chromatographic method does not replace a chemical analysis of the fractions, which would be a long, drawn-out and difficult procedure. It allows, however, the interpretation of the chromatographic picture, which shows distinct differences in colors and forms as related to qualitative and biological values. The causative chemical reactions (in most cases we

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have to do with a mixture of substances that cannot be separated easily) might be determined by other comparative methods insofar as this is possible today. To report this is not the purpose of the present article.

The separation takes place through the capillarity of the filter paper. This must have a homogeneous fiber structure in all directions. Whatman Filter Paper No. 1, chromatographic quality, is satisfactory. We use circular disks of 6″ diameter. The technical details of this method have already been described in BIO-DYNAMICS, No. 49: *The Art and Science of Composting*, E. E. Pfeiffer, p. 15-20.

In order to work out the chemical and biochemical details, one would have to separate sufficient amounts of the mixtures or extracts to be tested, using chromatographic absorption columns with various absorbents; the individual fractions are then collected and enriched, re-dissolved and then made available to further methods of analysis. This is not the subject of our present treatise. Simple circular chromatography as described here suffices for a quick orientation.

The same method has been applied for quality tests of aromatic or other active substances in coffee, tea, tobacco, extracts from drugs, foodstuffs, vitamin preparations, where chemical analyses were too complicated or did not show any results (as, for instance, in the coffee aroma of various kinds of coffee beans in various roasting processes).

We shall now discuss some of the results obtained.

OBSERVATIONS ON CHROMATOGRAM:

Preliminary Remarks: Even the best color reproductions do not always give the finest differentiation and hues of the originals. We are also aware of the fact that a verbal description is inadequate. Anyone who works with chromatograms himself will understand at once what is meant. In order to learn how to interpret the pictures, one starts with chromatograms of well-defined substances. Gradually a collection of "standards" will accumulate.

The following points have to be considered in reading the chromatogram:

(a) Number, width and color of the different zones, as well as their regular or irregular formation and shading.

In the pictures reproduced here in the insert, we distinguish three main zones: an outer and a middle zone, which are mainly

due to the organic material to be tested; an inner zone which indicates the presence or lack of mineralization. The width of the zones corresponds to the amounts of characteristic substances.

(b) Ring formations between the middle and outer zone and at the edge of the outer zone.

(c) Color of the zones: a light to medium brown, evenly distributed, points to a good colloidal humus formation; dark brown enclosures point to acid humus substances; violet radiations point to increasing mineralization and reduced organic substance. In the case of plant extracts, vitamin preparations and foodstuffs, other colors are observed. These will be discussed later on.

(d) Radiation, number, color and shape of spike-like formations. The violet radiations of the inner zone again indicate the breaking-down tendency toward mineralization. The various phases of fermentation (first, decomposition; second, humus formation; third, mineralization and too far advanced decomposition) are clearly indicated in the chromatograms of soils and compost.

Interpretation of the Pictures in the Insert:

For the sake of comparison, we first show some extremes of soil extracts (No. 1-6). Then we show chromatograms of composts from city garbage and manure with and without addition of the B.D. Compost Starter. Further, we show pictures of composts of the most varied kinds and origins, some of which have been produced with the help of the B.D. Compost Starter (No. 7-12). These reproductions illustrate the manifold possibilities of applying the method.

No. 10, 11 and 12 show various stages of industrial composting of poultry manure; first the raw material, then two stages of transformation. The procedure described here serves as a production control as used by the composting plant in question.

Wherever available, the results of analysis are reported. All figures of available minerals in soils are reported in lbs./acre. Organic matter is reported in percent.

Illustrations:

No. 1: An extract of black virgin soil from the Missouri River region in the State of Missouri. This soil possesses a natural, stable humus, an ideal, friable structure, and is of maximum fertility. Attention is drawn to the medium-brown

edge zone of the chromatogram with darker brown spots. The middle zone extends, spike-like, into the edge zone. The pattern of the radiating forms is harmonious. The inner zone is dark-brown; there is no violet coloring. Analysis of the soil: pH 7.0; available minerals: nitrate 20, ammonia 5, phosphate 170, potassium 180, exchange calcium 5500, organic matter 5%.

No. 2: As a contrast, we show a black, tough clay adobe soil from California. This soil is caked and crusted when dry, sticky and smeary when wet. It lacks aeration and offers many structural problems. The analysis of available minerals is misleading because this occluded soil was rather infertile. Its microflora was poorly developed. Plant roots could not utilize the available minerals. Analysis: pH 8.2, nitrate 64, ammonia traces, phosphate 30, potassium 450, exchange calcium 3000, organic matter 2.2%. The absence of valuable humus compounds is shown by the lack of form and faint brown coloration of the edge and middle zone of the chromatogram. The inner zone is comparatively large and contains hardly any humus signs. The capillary radiation is of a violet shade, indicating the mineralization of this soil. Its black color does not reflect the presence of stable humus but is caused by reduced crude organic matter. This soil is rather dead.

No. 3: A heavy, brown clay soil from a wet, badly drained meadow of a valley in the southeast of the State of New York, with a comparatively good stand of grass but many acid grasses and a weak stand of clover. The raw humus was acid. Observe the absence of the outer zone, which would indicate colloidal stable humus formation. Analysis: pH 5.4, nitrate 72, ammonia 0, phosphate 120, potassium 100, calcium traces, organic matter 2.8%.

No. 4: A peculiar "black dirt" soil from the southeast of the State of New York. This is an alluvial soil, probably of a bottom basin formed after the last ice age. It has a high content of organic matter and an unusually high content of nitrogen. It is, however, not a muck or peat soil. The fertility is high. The soil is well aerated. Chiefly vegetables are raised on it. Analysis: pH 6.8, nitrate 128, ammonia 5, phosphate 75, potassium 130, calcium 4000, organic matter 36.5%, total nitrogen (on dry base) 1.56%. A good, stable and colloidal humus condition is present.

No. 5: A medium heavy, good pasture soil, somewhat waterlogged at times but with a reasonably good humus forma-

tion. This soil has been improved over a four year period from a pH of 5.5 to 6.0, from an organic matter content of 2.8% to 4.5%, nitrates from 30 to 64 lbs./acre. The edge zone of the chromatogram indicates the degree of humus formation; the middle zone and spikes show the influence of the incomplete drainage to a mild degree. Analysis: pH 6.0, nitrate 64, ammonia 0, phosphate 140, potash 100, calcium low, organic matter 4.5%.

No. 6: A well aerated, well drained soil of the same farm in southeastern New York State. This field has always given reliable crops. The brown color of the edge and middle zone shows a slightly different but very significant hue, with less gray and more brown or yellow in it. Analysis: pH 6.2, nitrate 20, ammonia 0, phosphate 170, potash 100, calcium 200, organic matter 4.2%.

These two soils were under bio-dynamic treatment for many years. They show the improvement of the organic living condition.

(For the next following pictures, turn over to the last page of the folded illustration sheet.)

No. 7: Chromatogram from a compost made from a rather poor quality of city garbage, containing little organic wastes but lots of ashes and dust. The garbage was ground up and a pile set up for fermentation without addition of the B.D. Compost Starter. The fermentation period at the time the sample was taken was several months. The lack of a proper outer zone is noticeable on the chromatogram. The middle zone is gray-brown, with rather bulky, unformed spikes. The inner zone is large. Its violet radiation color is an indication of the high amount of mineral matter in this compost. This compost is truly "mineralized".

No. 8: Exactly the same garbage, set up at the same time as the previously described pile (No. 7) but with the addition of B.D. Compost Starter. The piles otherwise have been handled alike. The samples were taken within the same hour and both piles were the same age. We notice in the second chromatogram the presence of the outer, light brown edge zone, indicating good humus formation. The spikes of the middle zone are clearly formed and show a lively brown, not gray-brown color. The inner, mineralized zone is slightly smaller and has a slight brownish hue over the violet radiation, indicating that the mineralization has not advanced quite as far as in No. 7.

The analysis, on dry basis, was:

	No. 7	*No. 8*
pH	7.8	7.9
Total organic matter	46.2%	37.4%
Total nitrogen	0.77%	1.0%
Total phosphate (as P_2O_5)	0.80%	0.84%
Total potash (as K_2O)	0.80%	0.85%

Although sample No. 8 contained less organic matter, it contained more nitrogen. Its state of humus, of microlife and its biological value were considerably better, which is also indicated by the higher number of humus-forming actinomycetes in it. Illustration No. 8 also indicates that this compost had been fermenting too long, because in a properly handled B.D. Starter treated compost, one can stop the fermentation much earlier, in fact, before the third phase of fermentation; that is, here the mineralization phase has started. If the fermentation is interrupted at the right time, the chromatogram should not show a violet inner zone at all.

It is in such cases as the two reported here that the circular chromatographic method will show distinct qualitative, biological differences where the chemical analysis will not give the proper answer. We have studied many such cases of chemical equality and biological difference of compost but space and cost of reproduction do not permit presenting more examples. In all these cases, the composts which produced the more colorful, browner, better formed edge and middle zone and less of the violet radiation in the inner zone were also the ones which, in pot and field tests, produced greater amounts of yield, even if a lesser amount per acre was applied. The composts showing less colorful and less formed chromatograms — no matter what else the chemical analysis may be — produced inferior results in field tests.

No. 9: The chromatogram was made from a properly made and B.D. Starter treated compost composed of barnyard manure 60%, coffee waste 30% and soil 10%, including a small amount of garbage. There we see an almost ideal humus formation with an intensively brown colored edge and middle zone, well formed small spikes; even the inner zone is still brown and does not as yet show the violet radiation. The mineralization phase of the compost has not yet started. This compost was produced at Golden Acres Farm, Inc., Newtown, Pa. Analysis: pH 7.1, organic matter 26.8%, total nitrogen 2.04%, both on dry base.

No. 10: Chromatogram of fresh broiler-poultry manure as it arrives at the compost plant of M. P. C. Corporation, Gainesville, Ga. This poultry manure is collected from many poultry farms and contains the droppings plus a large amount of sawdust or peanut shell litter. The collected, mixed sample, obtained by quartering, is ground up in a laboratory mill and 5 gr. are extracted with 50 ml. of a 1% sodium hydroxide solution, of which 5 ml. are used for each chromatogram. The illustration shows that we deal with crude organic matter and not compost or humus.

No. 11: Chromatogram from composted broiler-poultry manure, that is, from the material as shown under No. 10. The compost is made by mixing said poultry manure (70%) with Hybro-Tite (granite meal of the Potash Rock Company of America, Lithonia, Ga.) 20% and colloidal phosphate 5% (Lonfosco of Loncala Phosphate Co., High Springs, Fla.) and alfalfa from screenings 5%, all by weight. The B.D. Compost Starter is added while mixing with a Barber Green Overhead Loader, Model OSM544 with snow buckets. The mixed material is piled on fermentation piles. The actual fermentation is completed in from 16-21 days, or even less under favorable conditions. It is important, when using our new hot and fast fermentation, that the piles do not over-ferment but are interrupted at the right moment, when the humus formation is well on its way and the mineralization (phase III) has not yet started. To control the composting process with the method described has become an important practice, especially if one wants to preserve the original nitrogen content and to obtain a good, saleable, organic humus fertilizer. This illustration shows a condition where the second fermentation phase has already been overshot and the mineralization has moderately advanced. We see, therefore, the broad edge zone (humus), the well formed middle zone, both already with a grayish-brown color and the violet inner zone, still with a brownish hue. A violet mineralized zone in this case is to be expected because of the addition of natural rock mineral to the original mixture. The compost at this stage smells and looks earthy.

No. 12: Compost from the same material, mixture and procedure as outlined under No. 11, but not quite as far advanced into the mineralization process. The very broad and intensively brown edge zone color indicates the presence of a very good, stable humus. The middle zone, though small but still with the

brownish, not grayish, hue indicates the transitional state between the second and third fermentation phase. This compost pile could have been used for drying and bagging already several days prior to the collecting of the sample. The violet inner radiation, still with a slight light brown hue, indicates the beginning of mineralization, as well as the presence of strictly mineral matter. Our advice in this case was to stop the fermentation at an even earlier stage. Analysis:

	No. 11	*No. 12*
pH	7.8	8.0
Organic Matter	15.0%	24.2%
Total nitrogen	0.53%	1.1%
Total phosphate (as P_2O_5)	3.30%	3.1%
Total Potash (as K_2O)	0.90%	1.0%

The illustrations so far demonstrate examples of biological values which cannot be caught in a purely chemical analysis. The circular chromatographic method lends itself to many other problems. In the next chapter a few other applications will be discussed.

II. Studies of Vitamin Preparations

The question has frequently been asked: "Is there a difference between a natural and a synthetic vitamin?" This question cannot be answered directly because, if we have a pure, crystalline vitamin of a well established chemical formula, this crystalline, chemically purest, product will be the same whether it came from a natural or a synthetic source. Such is the definition of a chemically pure substance. There will, however, be a certain difference which we may ascribe to the strictly chemical environment of the more or less (usually less) chemically pure and crystalline product. This may be due to so-called impurities or to concomitant association with unseparable by-products. Commercial folic acid preparations contain numerous impurities which, according to Drs. Warwick Sakami and Robert Knowles of Western Reserve University School of Medicine, Cleveland (Science, Jan. 30, 1959) have an "intense effect on certain enzymes" in the body.

In an entirely different realm, that of metals, for instance silverware or gold or other metal jewelry or steel, one can recognize the "imprint" of the manufacturer by the presence of certain trace mineral impurities which occur in one smelting plant and not in another. The different origin of such silver or gold can then be recognized to a certain extent.

It is also possible for so-called vitamin preparations to be typed according to their origin — natural or synthetic. This means, not that they are identical or not identical, but that the case history of extraction from original source material is reflected in the final product. Of course, the biological effect of these preparations would be similar.

In order not to be misunderstood, we will therefore use two terms in the following: (1) vitamin as a collective term — a combination of various factors (or substances) showing that particular vitamin reaction in a biological assay; (2) pure vitamin as defined by the exact chemical name, which may also mean a synthetically pure substance. We will therefore speak of a vitamin C preparation, of natural origin whatever this may be, containing the proper potency of C (definition 1) or of pure crystalline ascorbic acid, which may be synthetic or purified to the utmost possible degree (definition 2).

The circular chromatographic method will make this differentiation possible. It is, then well understood: the vitamin product (definition 1) will show a pattern with the inclusion of that which remained from the natural environment, while the pure preparation will not show the remnant of a natural chemical environment. In this regard, there may be a difference between the natural and pure vitamin (cf. illustrations No. 13 through 19, 21, 22).

Another question can be asked in this connection: To what extent have properties of the natural source material been preserved or removed in the manufacturing process? Or, in layman's words: To what extent has nature been refined and left out in a given product? This problem can be compared with the differences between an unrefined sugar cane juice, molasses, brown sugar and white sugar; or a refined oil as compared with an unrefined one. Obviously, the super-refined pure substance contains nothing but itself, while the intermediate steps of extraction or refinement may contain biological values in addition to the one proper only to the pure substance. This same "fading out" of concomitant naturally contained substances can be observed in the decline of nutritional value from whole grain (wheat, for instance) to bran, 90, 80, 70% extraction flour. This decline can be measured in terms of % protein, amino acid pattern, contents of minerals, trace minerals, vitamins and enzymes.

Again, an example from another realm may illustrate what we mean: Pure corn, olive or other plant oils will all have the same caloric value, namely, 884 calories per 100 gram and 100% fat, but unrefined, red palm oil may contain 100% fat and the same calories, namely 884 calories but, in addition, other intrinsic values, such as 10,080 I. U. of vitamin A, not even considering the differentiation between saturated and unsaturated fatty acids.

We have to ask our readers to keep the two definitions clear and apart so that they will get an accurate picture of what follows.

The method for the demonstration of differences in vitamin preparations is again the same: circular filter paper chromatography. Two paths are open: to use the vitamin preparation (the eluent) first and the developer (in our case, 0.5% of a nitrate of silver solution) second, or vice versa. In each case, one will have to try which approach leads to a better differentiation. For all illustrations of this report, the developer, nitrate of silver, was used first in order to impregnate or sensitize the filter paper, and the eluent followed. In all cases the vitamin and enzyme preparations were eluated with a 0.1% sodium hydroxide solution.

No. 13: Chromatogram of a chemically pure ascorbic acid U. S. P. We dissolved 0.1 gram in 10 ml. of 0.1% sodium hydroxide solution. After 30 minutes, the filter paper absorption was started. We observe a very small brown edge zone, one small undulated ring zone, two wide belts of the middle zone, all three showing pale shades of gray-violet, and a medium large yellow-brown inner zone.

No. 14: Chromatogram of a commercial product of ascorbic acid U. S.P. quality. Dilution and procedure as under No. 13. The pattern is slightly different; the inner ring of the middle zone is more brownish and the pale ring between the inner and outer ring of the middle zone is sharper; otherwise both patterns are quite similar.

No. 15: A vitamin C preparation, the vitamin C derived from Rose Hips. The manufacturer took specific care to preserve the "natural" quality and omitted purification to that degree which could be called "chemically pure." This pattern is quite different from the chemically pure one, showing much more undulation and spike formation, as well as radiation of the inner zone which, in fact, extends all the way out. The

middle zone shows a strong brown color; the inner zone contains a yellow-brown which had somewhat faded at the time the chromatogram was used for this reproduction. It is quite obvious that this natural product contains "something" which the pure chemical ascorbic acid did not show. This "something" consists of "impurities", in a manner of speaking, but they are by no means unessential or biologically without value. It is known, f.i., that natural forms of vitamin E complex lose up to 99% of their potency when separated from their natural synergists (Ann. Review Biochemistry 1943, p. 381). In fact, the "impurities" have a biological-dynamic importance. We know this because we have learned to recognize by many comparative chromatograms of living substrata, the undulating and radiating forms as well as the intensive colorations as a sign of biological activity, that is, of intrinsic values. At the present stage of our knowledge, it is not possible to identify "chemically" the nature of these concomitant substances, that is, the substances of the chemical or biological environment. We know, however, that the very same undulation and radiation, though with different colors, occur in enzyme studies and with extracts from living seed.

No. 16: Chromatogram of a natural vitamin C preparation by another manufacturer. Again, the manufacturer applied care to preserve the "natural" quality without going into the extremes of chemical refinement. This preparation was made from acerola. The eluation and procedure with these two preparations (No. 15 and 16) were exactly the same as in No. 13 and 14. All four preparations were diluted so that the same amount of ascorbic acid was present. This fourth preparation shows the ascorbic-acid-proper characteristic but, with regard to the concomitant "impurities" or biologically active accompanying ingredients, it is different from No. 15. Again, we see that intrinsic factors of biological activity are present. It is the presence of these biologically active factors, found in the natural but not the synthetic, which makes the chromatogram interesting. As with the soil, a chemical analysis for Ascorbic Acid (Vit. C) would be the same for No. 15 and 16 as for No. 13 and 14, but the chromatogram shows there is much more to the natural C than just Ascorbic Acid.

No. 17: Chromatogram of a chemically pure Thiamin Hydrochloride U. S. P. We dissolved 0.01 gram in 10 ml. of the 0.1% sodium hydroxide solution and proceeded as described

above. The edge zone is medium brown; the middle is medium large and gray, with two rings and an undulating separation toward the center; the inner zone is very large with a pink color in its center part, a strongly undulating separation towards the center and a white center ring.

No. 18: Chromatogram of a chemically pure Riboflavin U. S. P. We used 0.01 gram and the same procedure was applied as in all other cases. The outer zone shows a small, well separated brown ring, a medium dark gray middle zone (two rings) and an intensely yellow colored, homogeneous small inner zone, separated by a white ring from the middle zone.

No. 19: Chromatogram of a chemically pure Nicotinic Acid (Niacin) U. S.P. Again, 0.01 gram used, procedure the same as above. We see a pale brown, slightly undulated small outer ring. The middle zone, is separated from the almost colorless inner zone by a slight radiation gray-violet ring or halo. The middle zone shows several rings, the outer one dark brown with a reddish tinge, followed by a small halo, then a homogeneous medium brown-violet layer, again followed by a small "halo" ring.

These chromatograms of pure substances are necessary in order to establish "standard" patterns.

We will now discuss illustrations No. 21, 22 and 23, returning later to No. 20.

No. 21: A commercial product of a B Complex, containing refined sources of thiamin hydrochloride (0.3 mg.) riboflavin (0.1 mg.), nicotinamide (0.66 mg). The figures in parenthesis are the amounts used for the chromatograms. This suspension also contained pyridoxine chloride (0.02 mg.) and pantothenic acid (0.55 mg.). The difficulty here is that it is not possible to type such mixtures exactly to the same amount of concentration as is possible with the pure refined chemicals. In any case, we receive a mixed pattern, divided in the outer, double middle and inner zones. The outer and middle zones show the characteristics of the pure thiamin and niacin pattern, but the inner zone fails to show the intense coloration of the pure riboflavin, while it still shows the pink of thiamin. Chromatograms of the pure chemicals made with exactly the same concentrations as of the mixture have been made but cannot be published here because of lack of space.

No. 22: A Vitamin B Complex preparation made from natural sources with preservation of the original natural

quality. This mixture is composed somewhat differently from No. 21 and therefore cannot be compared exactly, one with the other, with regard to quantitative contents. The purpose of showing this pattern is to demonstrate again the presence of other biological factors as they become visible by the undulation and spikes. Again, the components of this preparation have not been refined to chemical purity but were properly typed as to their contents in specific vitamin potency. The eluation solution contained 0.3 mg. thiamin, 0.6 mg. riboflavin, 0.4 mg. niacin. In this case, the riboflavin (0.6 against 0.1 in No. 21) kicked through with a slightly yellow inner zone.

As we will demonstrate in another paper in the future, the specific "spoke" pattern is significant for enzymatic and other biological activity too, as one can observe in living tissue and germinating seed tissue extracts.

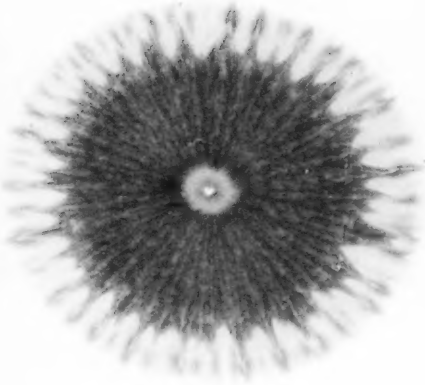
No. 23: Chromatogram of another Vitamin B Complex preparation made by the same manufacturer, who cares especially for the preservation of natural quality. Again, the vitamins were not refined to chemical purity and the preparation contains other biologically active materials. The formula is the same as the previous one, with the exception of niacin, of which 0.01 mg. were present in the aliquot specimen. The outer ring here is interrupted because of the stronger undulating pattern of the middle zone. The brown colors are a little darker, the yellow inner zone slightly fainter. As a whole, the pattern is about the same as in No. 22, pointing again to a strongly active biological quality.

We return now to No. 20. Illustrations No. 20 and 24 belong to investigations of enzymes, a study which is still going on.

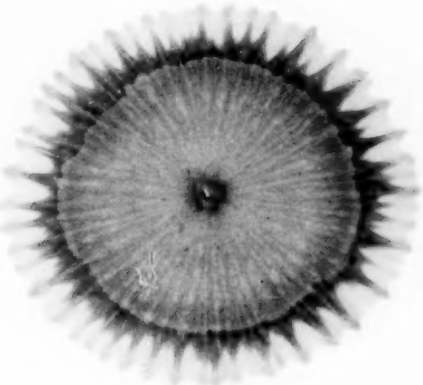
No. 20: Chromatogram of pure Diastase "Clarase" standardized. Diastase is a starch digesting, amylolytic enzyme. Its strong reactivity is indicated by the strongly undulating wavy middle zone. Here even the process of refining the enzyme to standard purity has not destroyed that pattern which we consider to be significant for biological activity in addition to the concomitant chemical nature of the investigated substance. Use was made of 0.1 gr. of clarase, diluted in 10 ml. of an 0.1% solution of sodium hydroxide (as eluent). About 1.5 ml. of the solution are taken up by the chromatogram.

No. 24: Chromatogram of pure Papain, a proteolytic enzyme obtained from the fruit of papaya. 0.1 gr. papain

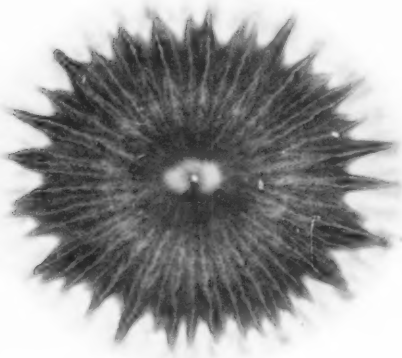
CHROMATOGRAMS OF SOILS



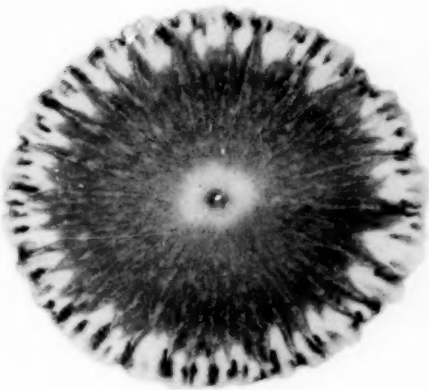
#1. VIRGIN SOIL, VERY FERTILE.



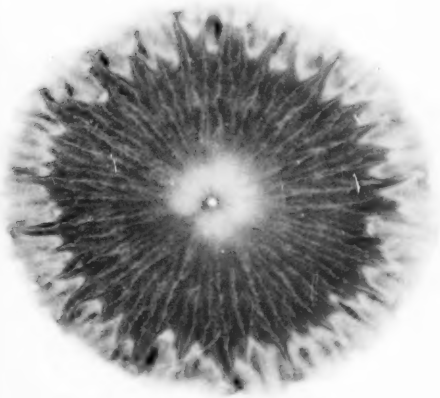
#2. ADOBE SOIL, DEAD.



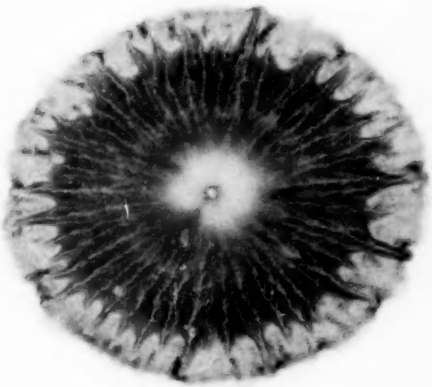
#3. BOTTOM LAND SOIL, MEDIUM FERTILE, WATERLOGGED.



#4. "BLACK DIRT", VERY FERTILE.

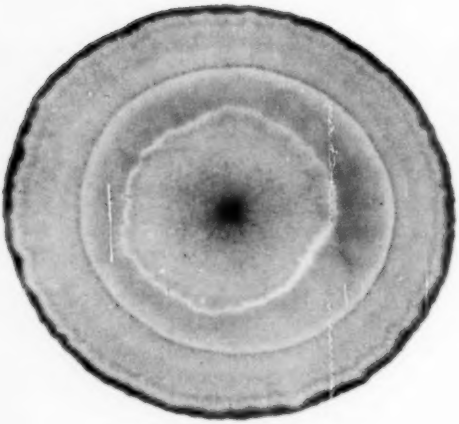


#5. FERTILE ORGANIC PASTURE SOIL.

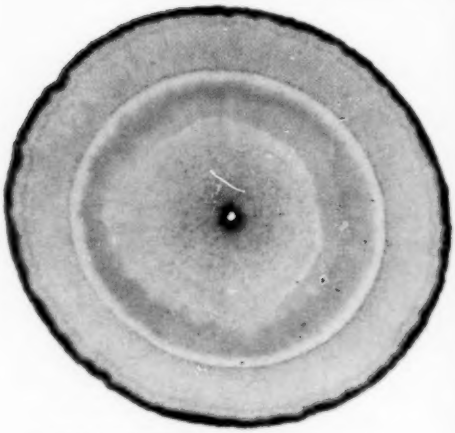


#6. WELL AERATED B. D. SOIL.

THE ILLUSTRATIONS ARE APPROX. ONE HALF OF NATURAL SIZE.

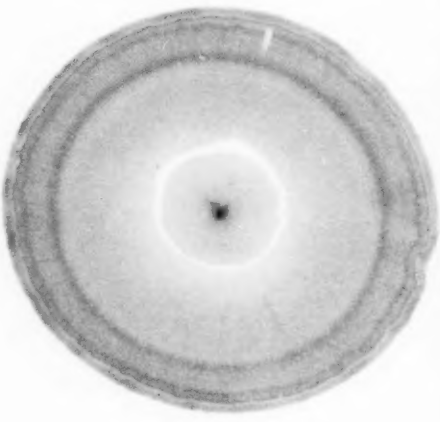


#13. PURE ASCORBIC ACID.



#14. A COMMERCIAL VITAMIN C PRODUCT.

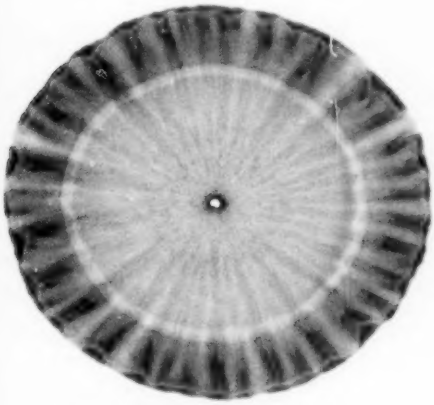
#17. PURE THIAMIN.



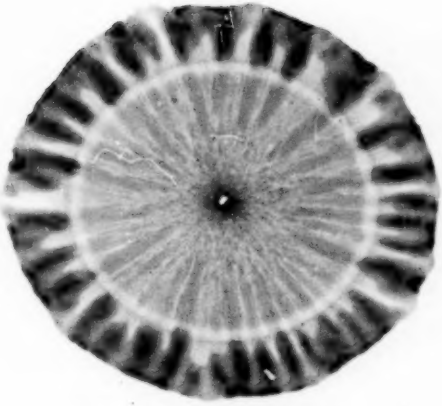
#18. PURE RIBOFLAVIN.

#21. A COMMERCIAL B COMPLEX PRODUCT.

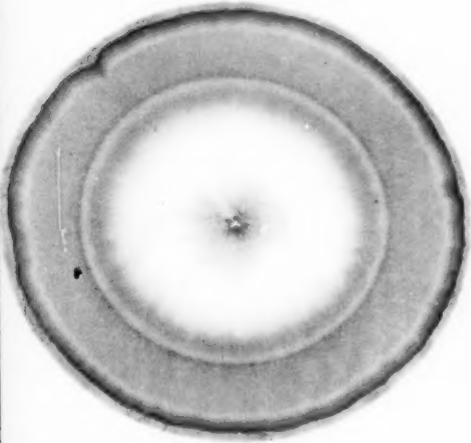
#22. AN ALLEGED NATURAL B COMPLEX PRODUCT.



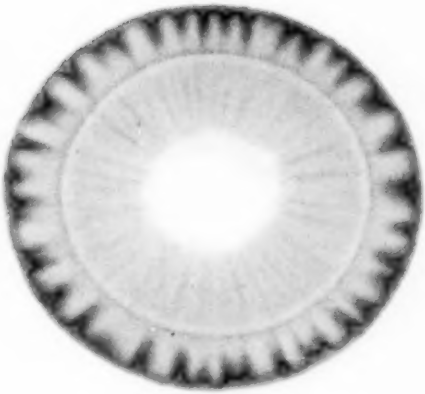
#15. A NATURAL VITAMIN C PRODUCT FROM ROSE HIPS.



#16. ANOTHER NATURAL VITAMIN C PRODUCT FROM ACEROLA.



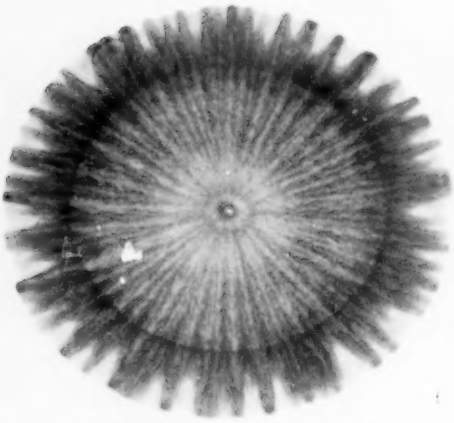
#19. PURE NIACIN.



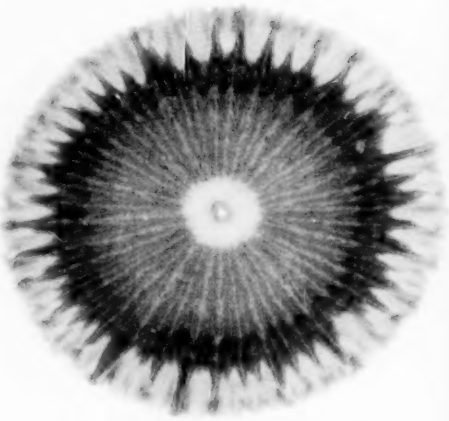
#20. PURE DIASTASE FROM CLARASE, A STARCH HYDROLYZING ENZYME.

#23. A NATURAL VITAMIN B COMPLEX PRODUCT.

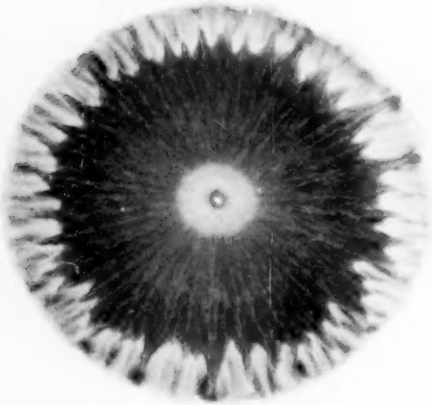
#24. PURE PAPAIN, A PROTEOLYTIC ENZYME.



#7. COMPOST FROM POOR CITY GARBAGE WITHOUT B. D. TREATMENT.

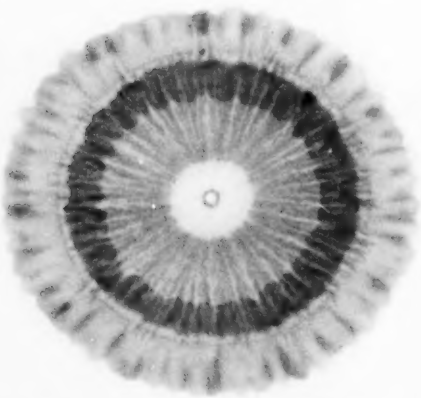


#8. COMPOST FROM SAME GARBAGE BUT WITH B. D. TREATMENT.

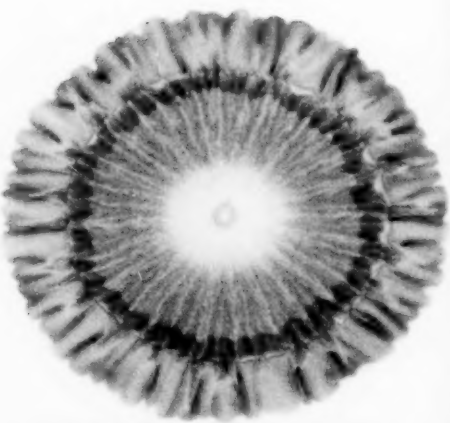


#9. COMPOST FROM BARNYARD MANURE AND COFFEE WASTE. B. D. TREATMENT.

#10. RAW BROILER POULTRY MANURE. UNTREATED.



#11. COMPOST FROM POULTRY MANURE (SEE #10). B. D. TREATMENT. ADVANCED FERMENTATION.



#12. COMPOST FROM POULTRY MANURE (SEE ABOVE). B. D. TREATMENT.

PLATES AND PRINTING BY FAIRFIELD LITHO CORP., STAMFORD, CONN.

eluated in 10 ml. of 0.1% sodium hydroxide solution, 1.5 ml. being absorbed in the chromatogram. This is a most peculiar pattern and color, entirely different from all others shown in this paper. At present we have brought in the two enzyme chromatograms only to utilize the opportunity to have an illustration printed in color. The discussion of the underlying enzyme problem must be reserved for a future publication.

In this communication we have restricted ourselves to presenting mere facts and observed material, omitting details of identification and discussion of the underlying chemical problems. The purpose of this paper was only to demonstrate the possibilities of application of the method reported. The role of silver nitrate as the most suitable reagent, because of its reducing ability in weak alkaline solution in the presence of naturally occurring biological material, has been established in histochemistry when staining tissue in order to detect vitamins, especially C, in situ. (Cf. Biochemistry and Physiology of Nutrition, Vol. II, page 24 and following, by Geoffrey H. Bourne and George W. Kidder, Academic Press, Inc., New York, 1953.)

Special credit is to be given to Curt John Witt for his help and advice in obtaining the color reproductions and to Fairfield Lithograph Corporation, Stamford, Conn., for carrying out the technical work of these reproductions.

PROFESSIONALS DEBATE PESTICIDE EFFECTS

FRED HECKEL

The problem of the chlorinated hydrocarbons, of which DDT is the best-known example, is being increasingly debated — and occasionally with considerable warmth — in the scientific world. This was demonstrated at one of the sessions of the Twenty-fourth North American Wildlife Conference held in New York City early in March. The gathering, sponsored by the Wildlife Management Institute of Washington, D. C., was attended by leading scientists from all over the U. S. A. The sessions were, however, also open to the general public.

Of greatest interest to readers of Bio-Dynamics was a technical session on "Pesticides and Controls", and of all the papers presented there the one that also aroused the liveliest concern among the professionals present had to do with the current one hundred million dollar spraying of active heptachlor or dieldrin in granular form over tremendous areas of our Southern

States. These pesticides, being spread from airplanes at a rate of 2 pounds per acre, are described as from 10 to 20 times more poisonous to wildlife (i.e. this includes all warm-blooded life) than DDT. The paper, dealing with this governmental effort to stamp out the imported fire ant, was presented by Leslie L. Glasgow of the Department of Forestry of Louisiana State University at Baton Rouge. In the discussion of it there was a marked cleavage among the scientists who spoke.

Introducing his subject, Dr. Glasgow commented that the fire ant does have an irritating sting and that the mounds it builds in their fields are definitely a nuisance to farmers. When these spring up, sometimes as many as 150 to the acre in a relatively short time, "it is naturally disturbing to the farmer", he said. So, in the autumn of 1957, a program was begun in the endeavor to stamp out the fire ant. It is a project of the Pest Control Division of the U. S. Department of Agriculture, with the costs being shared by the Federal Government and the various States and, in most instances, the local landowners.

The hundred million dollar cost, he referred to as "a mighty staggering sum, for which there is very little research". He went on to say that while in Louisiana the farmers have accepted the program, there was a growing resistance to it because of an increase in the sugar cane borer following the use of heptachlor. In his prepared paper he also stated that other farmers were hesitant because of a suspected increase in the rice stink bug after these applications.

He expressed regret that there had appeared in the press statements implying that the fire ant was a hazard to wild life, although there is "no evidence" for this. Further, he said, farmers give conflicting reports as to the effects of the control program itself. He said he had talked to farmers whose names had been given him by control personnel as having reported no kill of wildlife.

"Yet", he went on to say, "they freely listed species and numbers killed when I talked to them. Others told me on my first interview that there had been no kill, yet when I returned a second time they said there had been plenty, and went into detail. So it is clear that when you are interviewing farmers you get conflicting pictures.

"It is a fact that in any carefully studied areas there has been a high wildlife loss following application of these pesticides".

Dr. Glasgow went on to underscore this point by presenting data on the subject from various scientists who had made first-hand studies. These reports, which included analysis of the remains, showed that with few exceptions, treatment of large areas resulted in a high wild life mortality. On the other hand, he cited a Georgia study with a report of little loss of wild life. Also, data is lacking on the seriousness of sub-lethal doses.

"But we do know", he said, "that in areas which were treated the earthworm population has been reduced, and the earthworm survivors are carrying large amounts of heptachlor and its epoxide."

Another fact, he said, is that "careless application is a problem", as some of the planes working for the private contractors who do the actual application did not shut off the valves at flagged-out areas, etc.

In closing, he commented that "we have had wild life people who have exaggerated — which has not helped the situation any; and we have had control people giving out reports on the wild life situation. I think that if the present trend continues, toward a more rational approach, the public will have more respect and appreciation for both entomologists and game biologists".

Challenged by one of the Government personnel present at the meeting for references in his paper to increases in the sugar cane borer and the rice stink bug ("What relation do these have to the fire ant control program?"), Dr. Glasgow smilingly conceded that he was not himself an entomologist, but, he said, he had specifically been told by entomologists at Louisiana State University that he should not hesitate to include these instances in his presentation. He cited a scientific paper published by one of these entomologists in a bulletin issued last December, which included a report on surveys showing a definite increase of the sugar cane borer after the use of heptachlor in the treatment of fields. The data on the rice stink bug has not been published yet, he added, "but it is 99 to 1 that the fire ant control program has resulted in an increase of these bugs".

There had been statements from entomologists earlier in the discussion that beyond the loss of 2 calves there had been no authentic reports of losses of domestic cattle. Further, it was stated, test herds of "healthy animals" had been subjected to exposure and fed from treated pastures, and showed no ill effects. After the exchange regarding the alleged increase in

insect pests, a speaker who introduced himself on the microphone as "Peters, of Georgia" said he was "tired of hearing that there were no cattle, no livestock killed".

"It depends", he said, "on whom you want to believe. There have been too many reputable veterinarians who have certified the losses, and too many laboratory analyses of carcasses. These were not 'due to blackleg, or the effect of cold weather on emaciated cattle'. Somebody is covering up".

To which the chairman replied, in closing the discussion, by referring to the experiment in which a herd was exposed to treatment "with no harm", and said that "we have no data on half-starved animals".

One note of unanimity ran through the differences between the participants at the session; the need for a good deal more research and field study of all aspects of the problem. Thus, in the first paper of the lot, "Pest Control in Modern Setting", by Harlow B. Mills of the Illinois Natural History Survey, Urbana, Ill., the speaker warned, for instance, of the dangers of extrapolating the results of experiments on penned animals to wildlife in the woods and fields. He said we were in an era in which poisons of all kinds were going to be more and more a part of our environment, increasing as our population increases.

"We need more, real data", Dr. Mills emphasized, and in closing he pointed to the need for ecological control of pests. When another of the scientists present asked, during the discussion of his paper, whether the large number of kills did not indicate that the remaining animals had sub-lethal doses that would be affecting their reproduction, he replied that "we do not *know*". When another reported that bobwhites in a particular test area, not reproducing earlier in the year, were now again doing so, a speaker who introduced himself at the microphone as "Codum, of Texas", commented that "I think it would be safer to assume that there might be damages, rather than assuming that the whole thing is going to be safer than it *might* be".

The need for more, carefully planned research was also emphasized in a paper by Robert D. Chisholm, of the Agricultural Research Service of the U. S. D. A., at Moorestown, N. J.

"Hazards of Insecticide Residues" was the title of a paper by Norman Cannon of the Illinois Natural History Survey. He cited various eminent authorities who agreed that there were

no hazards in the Fire Ant program if the application was done as directed, although carelessness might do damage. He conceded that concern was rightly expressed by the wildlife people. He said there were some damages, but these were the result of careless application, and relatively not great. The users of pesticides, he felt, were "in duty bound to select the material that will do the job and yet be as harmless as possible to warmbloods".

Dr. Cannon closed with the comment that "There is no problem in determining that unfortunate incidents have occurred. The problem consists in determining the reasons for the unfortunate incidents, and trying to avoid their recurrence".

In the discussion after this paper, one of the speakers asked why, if heptachlor was ten times stronger than DDT, it was nevertheless applied at a rate of 2 lbs. per acre. Another asked whether there had been any studies of the effect on earthworms and other soil organisms. Others insisted that studies had shown no evidence of DDT killing earthworms that absorb it or killing the birds that eat the earthworms. Asked to comment on these matters, Dr. Cannon said that when the chlorinated hydrocarbons got into the soil it was like putting them into a can, and they persisted for an extremely long time. A Government entomologist present stressed that "The Fire Ant Control Program was demanded by Congress, not initiated by the Department of Agriculture". He said studies were now going on and "it is likely that we will recommend less heptachlor per acre in a relatively short time".

In a paper dealing with pollutional effects, Clarence M. Tarzwell of the U. S. Public Health Service, Cincinnati, Ohio, remarked that as water is going to have to be re-used, it means that "users have to turn it back so that the next user can use it". He said that "speaking generally, the organic phosphates are not as toxic as the chlorinated hydrocarbons".

"I'm worried a little about ground water contamination", he said. "The fact is that we need a good deal of investigation. I would suggest that we look for insecticides that are specific for the organism that is to be controlled. I think we'll have to come to this if our water resources are to be preserved."

The final paper of the series directly concerned with insect control was entitled "Pesticide-Wildlife Problems and Research Needs". The speaker, Daniel L. Leedy of the U. S. Fish and Wildlife Service, Washington, D. C., said that some progress

had been made on the research side "but we have a long way to go". Research, he said, "should occur *prior to* large scale control operations".

AN INDUSTRIAL ITEM

The following, from the Market Newsletter of the publication Chemical Week, *issue of March 21st, 1959, is of definite interest in connection with the discussion reported above.*

Heptachlor pesticide is under fire by Southern farmers who — in collaboration with the U. S. Department of Agriculture's mass spraying program — have been using the chemical to fight the South's fire-ant scourge (CW, Feb. 8, p. 97). Louisiana sugar-cane fields treated with heptachlor, it's claimed, show 27.81% greater infestation by the sugar cane borer than is found in untreated fields. Reason: the pest's natural enemies are killed off by the heptachlor. The farmers' complaints are borne out by two independent surveys: one by the U. S. Department of Agriculture, the other by a Louisiana State University research group.

Several state and university groups will study the charges further. If their findings further corroborate the current charge, a special committee — headed by Louisiana Agricultural Extension entomologist Kirby Cockerham and including Antoine Dalfrey, state supervisor of the fire-ant program — will likely push for these corrective measures: declare affected cane farms emergency areas and provide funds for a borer control program; provide restitution for current and future losses incurred through use of heptachlor; study the residual effect of heptachlor for at least a three-year period.

Spokesmen for the pesticide industry admit that the government's fire-ant program is already in trouble, and the experience of the Louisiana farmers provides opponents of mass spraying programs with the kind of ammunition they seek. If the fire-ant program experiences a setback, it will obviously mean cutbacks in heptachlor demand and, perhaps, catalyze similar curtailments of other spray programs that use other types of pesticides.

THE ANNUAL B.D. CONFERENCE

New developments in Biodynamics, and the chance to share and swap experiences with others, will again be available to those who attend the Annual Bio-Dynamic Association Conference at Threefold Farm, Spring Valley, N. Y., August 22nd and 23rd. The program, already fairly well jelled at the time of this writing, includes time for questions and discussion (with Dr. Pfeiffer in the chair), practical demonstrations, first-hand reports by representative B.D. farmers and gardeners, as well as interesting lectures.

Among the latter are two by Dr. Pfeiffer. On Saturday evening he will present his newest findings in the field of "Food Quality and Food Testing", and Sunday morning he will report on "The World Agriculture and Food Situation".

The sessions start at 9 A. M. Saturday, with a lecture, the first in several years, by Evelyn Speiden Gregg, long our secretary-treasurer. In 1958 she had just returned from India and took part in a panel on nutritional problems in that country. Her talk will be followed by Russel J. Keep, Jr., and Walter Stuber, speaking on "Co-operative Marketing of Bio-Dynamic Products". In the afternoon Alice Heckel speaks on "Working with Nature for Pest Control". Later there will be a tree paste and mulch demonstration. While some are already familiar with the new developments in regard to the tree paste, those who are not, can get a lot from seeing the practical application of this aid to B.D. practice.

The Sunday sessions start at 9 A. M. with a lecture by Heinz Grotzke of Golden Acres Farm. The annual B.D. Association Meeting on Sunday afternoon will be followed by the farm and garden reports. At this session Dr. E. F. Winter, that devoted and successful B.D. Homesteader who is familiar to all who have been attending these sessions in recent years, will speak on "Intensifying Our B.D. Homestead Production". Hans Mahle will report on "Observing Animals". Walter Stuber, Ludwig Piening, Ernst Fetzer, Ernest Daniel, Walter Buschman and John E. Volkert are among those who, it is hoped, will present reports on their results and observations.

The question and discussion period, always one of the most valuable parts of these annual conferences, will be held on Sunday evening.

Registration for the Conference ($4.00 registration fee), will be started by our secretary-treasurer, Mrs. Harris Porter,

on Friday evening for those who arrive early, and continued at 8 A. M. Saturday. Admission to single sessions will be 75c.

For advance reservations for room and meals, address Mrs. Richard Kroth, Threefold Farm, Spring Valley, N. Y., enclosing $5.00 deposit. Experience has shown that if you intend to come, this is a wise precaution, as the place usually fills up well in advance of the Conference. Last year the attendance was so heavy that late comers could not even be served meals.

F. H.

BOOK REVIEWS

From The A-Horizon To Zygopetalum

This 1145 page ENCYCLOPEDIA OF ORGANIC GARDENING compiled during the last ten years by Mr. J. I. Rodale and his Staff was published in April by Rodale Books, Inc., Emmaus, Penna. It is available in two editions, the regular at $7, a thumb-indexed volume at $8.

The problems faced by a reviewer when contemplating a book of this sort which covers so much ground are really beyond description. Where to start, what spots to hit in between, where to end; these are but a few of the pertinent questions. Incidentally, the A Horizon mentioned in the title refers to the upper horizon of the soil, the other horizons, B and C, are dealt with in the encyclopedia in their corresponding alphabetical order. As for Zygopetalum, it is an epiphytic orchid and the last item treated in the book. Most of the plants are listed under well-known popular names rather than their botanical designations; however, their Latin names are also given in each article in order to avoid confusion. An excellent system of cross-references enables the reader to find material related to the specific listing which he has first referred to.

There are articles on soils, composting, humus, organic matter, orchards, shrubs, vegetable gardening, diagrams, tables, photographs, a wealth of material which will probably take at least as many years to assimilate as it took to compile. There is one complaint which it seems necessary to voice just here and that is the absence of the names of the publishers of various authoritative books which are quoted from time to time. The user of the encyclopedia might like to study these books during the long winter evenings, and this information would make

it easier for him to find them. However, this lack may be excused when one comprehends the mass of information that has been gathered together, including tables, one on the PERCENTAGE COMPOSITION OF VARIOUS MATERIALS, for instance, compiled by the U. S. Dept. of Agriculture, giving the NPK values of a host of materials; another listing plants suitable for different kinds of wild gardens; another listing herbs, and so on.

Biographical material about the various pioneers in the organic movement is also included in the volume. There is a long article about Sir Albert Howard, really the first one to work on the world-wide dissemination of information about the natural treatment of soils and plants. As many of our readers know, he developed a method of composting in India, known as the Indore method, the name deriving from the area in India where he worked it out. There is an article about Dr. Pfeiffer and one about the Bio-Dynamic Method. Both of these last articles are a little out of date, no mention being made of the developments in the application of the B. D. method which have taken place in the last 10 or 15 years. Old readers and members of the B.D. Association, of course, know about the development of the B.D. Starter for compost making and the B.D Tree Spray, and the many other results of Dr. Pfeiffer's research which have been published in this magazine. It is regrettable they were not mentioned so that others might have been given the opportunity of following up the references.

On the whole, it is an excellent practical reference book, written clearly and simply, and full of factual information of use to the gardener, male or female, organic or biodynamic, even to the ardent user of chemicals. Mr. Rodale and his staff deserve high praise for their labor and its results.

ALICE HECKEL

INDIAN FOODS AND NUTRITION

by MILDRED MCKIE KEITHAAN, M.D.
(Pas Press, POB 349, Madras 2, India.)

More than twenty years ago Dr. Mildred McKie, as she prefers to be called, went to India to practice medicine. However, after two years, she decided that it was not pills and injections that India needed, it was FOOD. Since then she has devoted much time and energy to studying, testing, experiment-

ing, cooking and teaching. To make the information which she gathered, available to all, she has written a small book. In the introduction Dr. McKie says, "There is a great need in India for more knowledge of foods and their nutritive value. The practical aspects and simple truths of nutrition may be easily understood, and should be learned by the rising generation not only that they may derive benefit for themselves but also that they may be in a position to instruct others."

This book was written for the laymen of India but the basic principles and information given in it can be used anywhere. Dr. McKie's chief concern seems to be to help the natives of India to help themselves to a better diet by utilizing the foods available and to preserve and prepare them in a manner that will not destroy their nutritive values. Again, quoting Dr. McKie, "At a rural center which I visited recently, where famine relief was being given, a woman came begging for food. I pointed out that the kera on which she was standing was not only edible but nutritious and the cooked juice could be fed to the baby. In that small garden a dozen edible plants were found, and the woman was helped to help herself. These sources are known to those who somehow manage to survive. More use should be made of that knowledge." Again and again Dr. McKie emphasizes the advantages of using natural foods, whole grains, fresh and raw fruits and vegetables and natural sweets. All these to be prepared in the way which best retains their nutritional values. She points out that many times after she had experimented and found the best way to prepare certain foods, she would find a grandmother in the village who would tell her that that was the way that she (the grandmother) used to prepare it. In the days when less preparation, less processing and refining were done the foods were more nutritious. The older generation also had an instinctive knowledge of the value of the wild plants. Dr. McKie urges that this knowledge be rediscovered.

Another point of interest is that many in India are restricted in their diets by their religion, so substitutes must be found for meat, eggs, etc. Nuts can easily be raised in India and their high food value is pointed out. A recipe is given for a milk made from ground nuts (peanuts) which has a nutritive value comparable with cow's milk. Simple instructions are given for ways in which the nutritive value of foods already in common usage may be increased. Some of these suggestions include the

sprouting, fermenting and malting of grains and exposing the vegetable oils to the sun for a few hours during mid-day to develop vitamin D in them.

One chapter is devoted to recipes. This chapter is begun with these words, "Many of the older women in India who are experienced in the art of Indian cooking would be able to prepare several hundred different dishes. These recipes which have been handed down from mother to daughter need to be collected and widely distributed, as they were properly prepared and seasoned."

The last twenty-five pages contain the names of different foodstuffs common to India and their equivalents in ten different Indian Languages. This part of the book of course meant nothing to this reviewer but it does make one realize the tremendous task Dr. McKie must have had in gathering and preparing the material for a book such as this.

Word has been received that Dr. McKie is expected to return to the United States this fall and that she hopes to do Bio-Dynamic farming on her Iowa farm. I do hope that we may have the pleasure of meeting her at a Bio-Dynamic conference one day soon.

RUTH EASTMAN

DATES OF 1959 FARM CONFERENCE

The annual Farm Conference at Golden Acres, Walter Stuber's farm at Newtown, Pennsylvania, will be held on Saturday and Sunday, October 17th and 18th. Details of the program will be announced later.

BOOKS RECEIVED

THE SCIENTIFIC PRINCIPLES OF CROP PROTECTION. By Hubert Martin, D. Sc. (Lond.), A.R.C.S., F.R.I.C., F.C.I.C. Edward Arnold (Publishers) Ltd. London. $12.50.

This is the 4th revised and enlarged edition of a book which was formerly known under the title Scientific Principles of Plant Protection. It can be obtained from St. Martin's Press Incorporated, 103 Park Avenue, New York 17, N. Y.

Dr. Martin, at present Director of the Science Service Laboratory, Canada Agriculture, at London, Ontario, has

gathered all of the material covered in this book from many sources and presents it as a detailed survey of the physico-chemical factors for the mycologist and entomologist and provides the chemist and physicist with material so that they can approach the biological side of the problems of plant protection. It comprises a masterly synthesis of the literature and points of view on the subject.

A. P. H.

FROM THE SECRETARY'S DESK

The printing of the Membership-Subscription folders was made possible through the kindness of two of our members. Mrs. Louis L. Browne made a financial donation and Miss Alma Lou Stansbury surprised us with a donation of 30 copies of "The Earth's Face and Human Destiny" by Dr. E. E. Pfeiffer. This book has been out of print for some time and we were indeed fortunate to get these copies which you may buy for $2.75 each, while they last.

The two basic books on Bio-Dynamic Farming and Gardening by Dr. Pfeiffer are still out of print. However, much information on the Bio-Dynamic method may be found in back issues of our quarterly, BIO-DYNAMICS and the following pamphlets:

The Biodynamic Treatment of Fruit Trees, Berries and Shrubs	.60
Companion Plants & Herbs	.50
BIO-DYNAMICS (quarterly) per yr.	1.50
single copy (current or back issue)	.40
all available back issues (40 issues)	8.50
package of 30 back issues	6.00
any 10 available back issues	3.00
Index BIO-DYNAMICS 1941-1955	.25
The Minister's Vegetable Garden by The Rev. John Philbrick and Helen Philbrick	.25

This pamphlet by the Philbricks is not strictly Bio-Dynamic but is written along Bio-Dynamic lines and is very helpful to the home gardener.

J. P.

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B. D. Rye Flour, Rye Cereal and Cornmeal

Organic Oatmeal, Cheddar Cheese

Made from Unpasteurized Milk

Send a Postal Card for Complete List, and Prices, to

WALTER BUSCHMAN

SUGAR LOAF ORANGE COUNTY, N. Y.

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